

## University of Groningen

### Norrie disease gene polymorphism in Indonesian infants with retinopathy of prematurity

Siswanto, J. Edy; Ronoatmodjo, Sudarto; Sitorus, Rita S.; Soemantri, Ag; Setijaningsih, Iswari; Sauer, Pieter J. J.

*Published in:*  
Bmj open ophthalmology

*DOI:*  
[10.1136/bmjophth-2018-000211](https://doi.org/10.1136/bmjophth-2018-000211)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2019

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Siswanto, J. E., Ronoatmodjo, S., Sitorus, R. S., Soemantri, A., Setijaningsih, I., & Sauer, P. J. J. (2019). Norrie disease gene polymorphism in Indonesian infants with retinopathy of prematurity. *Bmj open ophthalmology*, 4(1), [000211]. <https://doi.org/10.1136/bmjophth-2018-000211>

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

# Norrie disease gene polymorphism in Indonesian infants with retinopathy of prematurity

J Edy Siswanto,<sup>1</sup> Sudarto Ronoatmodjo,<sup>2</sup> Rita S Sitorus,<sup>3</sup> Ag Soemantri,<sup>4</sup> Iswari Setijaningsih,<sup>5</sup> Pieter J J Sauer<sup>6</sup>

**To cite:** Siswanto JE, Ronoatmodjo S, Sitorus RS, *et al.* Norrie disease gene polymorphism in Indonesian infants with retinopathy of prematurity. *BMJ Open Ophthalmology* 2019;**4**:e000211. doi:10.1136/bmjophth-2018-000211

Received 19 September 2018  
Revised 12 December 2018  
Accepted 20 December 2018



© Author(s) (or their employer(s)) 2019. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

<sup>1</sup>Neonatology, Harapan Kita Women and Children Hospital, Jakarta, Indonesia

<sup>2</sup>Epidemiology, University of Indonesia, School of Public Health, Depok, Indonesia

<sup>3</sup>Ophthalmology, Cipto Mangunkusumo Hospital, Jakarta, Indonesia

<sup>4</sup>Pediatric, Dr Kariadi General Hospital Medical Center, Semarang, Indonesia

<sup>5</sup>Eijkman Institute for Molecular Biology, Jakarta, Indonesia

<sup>6</sup>Pediatric, Universitair Medisch Centrum Groningen, Groningen, The Netherlands

## Correspondence to

Dr J Edy Siswanto; edyjo15@yahoo.com

## ABSTRACT

**Objective** Retinopathy of prematurity (ROP) is a major cause of blindness in newborn infants, which also occurs in low-income and middle-income countries. Why ROP progresses in some infants while it regresses in others is still presently unknown. Studies suggest that genetic factors might be involved. Mutations in the Norrie disease (ND) gene are suspected to be related to advanced ROP development. Indonesia is a country with relatively high incidence of ROP, yet the role of these genetic factors in the pathogenesis of ROP cases is still unknown. The study aimed to investigate the presence of mutations in ND on the X chromosome in infants with both non-advanced and advanced ROP in Indonesia.

**Methods and Analysis** This is a case-control study of polymorphisms in six variants within the ND gene in exon 3, C597A, L108P, R121W, A105T, V60E and C110G, in preterm newborn infants in four major hospitals in Greater Jakarta, Indonesia.

**Results** We included 162 preterm newborn infants. ROP was diagnosed in 83 infants, and 79 infants served as controls. Among those with ROP, 57 infants had type 2, while others had type 1. We did not find any gene polymorphisms in any of the infants with ROP nor in the control group.

**Conclusion** We conclude that it is very unlikely that the six polymorphisms in exon 3 of the ND gene studied in this paper are involved in the development or progression of ROP in preterm infants in our population sample in Indonesia.

## INTRODUCTION

Retinopathy of prematurity (ROP) is a major cause of blindness in preterm infants. The ROP incidence in infants with gestational ages of more than 26 weeks has been decreasing in developed countries due to improved quality of neonatal care. In contrast, an incidence is seen in infants born before 26 weeks.<sup>1 2</sup> We showed that the incidence of ROP is higher in preterm infants born in Indonesia as compared with developed countries, and is also seen in infants with gestational ages of up to 34 weeks.<sup>3</sup> Our results are in accordance with other studies in low-income and middle-income countries.<sup>4</sup>

## Key messages

### What is already known about this subject?

- ▶ Mutations in the Norrie disease (ND) gene are suspected to be related to advanced retinopathy of prematurity (ROP) development.
- ▶ Previous studies have shown inconsistent results regarding the mutations in the ND gene and the development or progression of ROP.
- ▶ There might be variations in the presence of mutations among different countries in Asia (previous studies from Korea and Japan in East Asia, and Kuwait as a Middle Eastern Asian country).

### What are the new findings?

- ▶ Mutations in the ND gene (especially in exon 3) play minimal to no role in the progression of severe ROP given the fact we did not find any polymorphism in any of the patients in Indonesia.

### How might these results change the focus of research or clinical practice?

- ▶ Our result cannot explain why severe ROP is more frequent in Indonesian infants than in infants from other racial backgrounds.
- ▶ Further studies must be conducted to find definitive explanations as to why ROP regresses in some patients, while it progresses in others.
- ▶ We have studied six gene mutations that are already known to be related to the development of ROP and no mutation was discovered in any of those genes.
- ▶ However, it is possible that the development or progression of ROP might be associated with other mutations in the ND gene beyond what we investigated in this study.

ROP is a multifactorial disease. Gestational age, birth weight and use of supplemental oxygen are known risk factors. In Indonesia, we found that other factors such as sepsis, asphyxia, patent ductus arteriosus and multiple blood transfusions might also play a role.<sup>3</sup> However, none of the presently identified risk factors can explain why some infants develop ROP while others do not. It is also not known why ROP regresses in most of the infants and progresses to severe forms in others.

A number of studies have suggested that genetic factors influence the development and severity of ROP.<sup>5–14</sup> A study of monozygotic and dizygotic twins showed that genetic factors accounted for 70% of the variance in liability for ROP.<sup>5</sup> Cooke *et al*<sup>13</sup> found that a mutation in the VEGF gene was related to the progression of ROP to threshold levels. Preterm infants who were homozygous for six alleles in the VEGF gene were twice as likely to develop threshold ROP.<sup>13</sup> Mohamed *et al*<sup>14</sup> studied the genetic polymorphisms with candidate genes and genes previously genotyped for associations with preterm delivery. They found that IHH, AGTR1, TBX5, CETP and GP1BA were all associated with the development of ROP.<sup>14</sup> Most studies described an association of mutations in the Norrie disease (ND) gene on the X chromosome with the progression of ROP.<sup>6–12</sup> However, other studies could not confirm these results. ND, familial exudative vitreoretinopathy (FEVR) and ROP are retinal diseases which share similar phenotypes. ND is caused by a mutation in the Norrie (ND) gene, a condition which leads to a deficiency of Norrie protein.<sup>15</sup> FEVR is characterised by reduced retinal vascularisation, retinal retraction and retinal detachment.

There are indications that racial differences exist in the development of ROP. Studies have shown a lower incidence of ROP in African-American infants and a higher incidence in Asian infants.<sup>16</sup> That race might be involved in the development and progression of ROP was also found in studies in different populations in the USA.<sup>17</sup>

No study has evaluated if the higher number of ROP incidence in Indonesia might be related to the presence of mutations in the ND gene. Therefore, we investigated if mutations in the ND are present in infants with ROP in Indonesia.

## METHODS

This is a case-control study conducted in four hospitals (Harapan Kita Women and Children Hospital, Budi Kemuliaan Hospital, Awal Bros Tangerang Hospital, Royal Taruma Hospital) in Greater Jakarta, Indonesia. All infants admitted to the neonatal intensive care unit of each hospital and screened for ROP were included in the study. Most patients came from Jakarta, and the rest from West Java, Central Java, Sumatra and Kalimantan. Infants with birth weight less than 1500 g and/or gestational age of less than 32 weeks, and infants with a higher birth weight and/or gestational ages who needed respiratory support, were screened for ROP and included in this study.

Infants diagnosed with ROP type 2 or type 1, according to the Early Treatment for Retinopathy of Prematurity criteria,<sup>18</sup> were included as patients, while infants without ROP were included as controls. A venous blood sample or a buccal swab was obtained from each infant.

## DNA analysis

The basis of this study were Haider *et al*'s<sup>19</sup> findings in 2002 that the incidence of AA genotype of the C597A

polymorphism in the ND gene was considerably higher in advanced-stage ROP cases. Therefore six alterations of the ND gene were analysed by amplifying the ND gene in the third exon using primers from these previous studies.<sup>7 19</sup> We followed the procedure, techniques and primers that have been used previously, PCR-RFLP (PCR-restriction fragment length polymorphism) for four genetic variants C597A, L108P, R121W and A105T, and PCR-SSP (PCR-single specific primer) for two other variants V60E and C110G.<sup>7 19–21</sup> DNA sequencing was performed on 36 samples to verify the results of PCR-RFLP and to detect V60E and C110G mutations where the PCR-SSP failed.

We did not use PCR-RFLP for the two additional variants due to the method and primers used in the findings of V60E and C110G polymorphisms in previous studies<sup>22</sup> (Torrente *et al*<sup>21</sup>) using PCR-SSP.<sup>21 22</sup> This method allows gene amplification where only a partial sequence information is available even when the information is only known at one end of the DNA fragment. We screened for six variants all within the same exon because some supporting literature states that the majority of mutations are seen in the 'translated region' in the third exon of the ND gene.<sup>23–26</sup> Although in subsequent studies other gene locations were shown that might be associated with ROP, we chose the above-mentioned six genes because they were evaluated in most of the studies. In table 1 we describe the studies on the relationship between ND gene mutations and ROP.

DNA was isolated from the patient's whole blood, buffy coat or buccal swab. DNA isolation was done using Isolation Kit Gene aid protocols. The DNA obtained from the PCR (DNA fragment sized 297 base pair [bp]) was cut with restriction enzymes to determine the alterations in exon 3. HaeIII restriction enzyme was used to detect C597A polymorphism and L108P mutation, MspI restriction enzyme for R121W mutation, and MboII restriction enzyme for A105T mutation. Subsequently, PCR-RFLP results were visualised by electrophoresis. Optimisation of PCR-SSP wild-type and mutant primer to detect V60E and C110G mutation was carried out using annealing temperatures of 50°C–72°C, primer concentrations of 25 pmol, 30 pmol, 35 pmol and 40 pmol, as well as the addition of dimethyl sulfoxide. Optimisation for the wild-type V60E primer was successfully performed (258 bp). The PCR-SSP for wild-type V60E was performed using an annealing temperature of 57°C and a primer concentration of 30 pmol or 40 pmol. However, optimisation of mutant V60E primer, wild-type and mutant C110G primer failed to get the optimal PCR condition. Results for the optimisation showed many unspecific bands. Wild-type and mutant C110G primer also produced thick dimers. Therefore we decided to perform DNA sequencing to verify the results of PCR-RFLP and PCR-SSP.

## RESULTS

A total of 182 infants were enrolled in this study. The DNA amplification was performed successfully in 162

**Table 1** Published studies on the relationship between mutations in the ND gene and ROP

Number	Researcher, population	Genetic polymorphism	Screened gene location	Number	Researcher, population	Genetic polymorphism	Screened gene location
1	Shastri <i>et al</i> , <sup>6</sup> USA	R121W and L108P missense mutations.	Exon 3	6	Haider <i>et al</i> , <sup>22</sup> Kuwait	A105T dan Val160Glu mutations.	Exon 3
2	Buffen <i>et al</i> , <sup>31</sup> (1999), USA	Mutations at 3' UTR.	Exon 1	7	Haider <i>et al</i> , <sup>19</sup> Kuwait	AA genotype of C597A polymorphism.	Exon 3
3	Haider <i>et al</i> , <sup>7</sup> Kuwait	R121W and L108P missense mutations.	Exon 3	8	Kim <i>et al</i> , <sup>9</sup> Korea	No gene mutation.	3 exon and their flanking areas
4	Talks <i>et al</i> , <sup>27</sup> London, UK	Deletions (one 5 bp and the other 71 bp) of the CT repeat sequence in exon 1 of NDP).	Exon 1	9	Hutcheson <i>et al</i> , <sup>28</sup> various ethnic groups in the USA	Six alterations: 1 in the 5' UTR of exon 2, and 4 in the 3' UTR of exon 3, and the other 14 bp deletion in the 5' UTR of exon 1.	Exons 1, 2, 3, 5' UTR exon 2 and 3' UTR exon 3
5	Hiraoka <i>et al</i> , <sup>11</sup> USA	Insertion of an additional 12 bp CT repeat and 14 bp deletion in exon 1.	3 exons, splice sites and the 3' UTR region	10	Hiraoka <i>et al</i> , <sup>12</sup> Japan	A heterozygous mutation at 5' UTR of exon 1 in the ND gene and a leucine insertion in the signal peptide of LRP5.	3 exon and their flanking areas

ND, Norrie disease; ROP, retinopathy of prematurity; UTR, untranslated region; bp, base pair.

samples. The clinical characteristics of the infants are presented in [table 2](#).

Birth weight and low socioeconomic status were slightly higher ( $p<0.05$ ) in the control group, while other characteristics were not different between the groups ( $p>0.05$ ). Of the infants with ROP, 24 had a birth weight of less than 1000 g and 15 a gestational age of less than 28 weeks. Forty-seven infants with ROP had a birth weight of 1000–1500 g and 43 infants a gestational age of 28–32 weeks. Twelve infants had a birth weight of more than 1500 g

and 25 infants had a gestational age of above 32 weeks ([table 3](#)). Fifty-seven infants showed type 2 ROP, while 26 showed type 1 ([table 3](#)).

The PCR-RFLP process showed that all samples have wild-type ND genes ([table 4](#)). Mutations or polymorphism of C597A, L108P, R121W and A105T were not found in any of the samples collected both from infants with ROP and the control group.

DNA sequencing was performed on 36 samples to verify the results of PCR-RFLP and to detect V60E and C110G mutations where the PCR-SSP failed. Samples from 26 infants with ROP and 10 infants from the control group (without ROP) were processed in DNA sequencing. Observed sequences were compared with sequences in GenBank using the BLAST program. The results of BLAST showed 99% homology with *Homo sapiens*' ND,

**Table 2** Clinical characteristic of infants

Clinical characteristics	ROP positive (cases)	ROP negative (control)
Total (n)	83	79
Birth weight (g)	1182 (529–2430)	1331 (600–2100)
Gestational age (weeks)	30.5 (25–38)	31 (24–35)
Male (%)	38 (45.8)	32 (40.5)
Multiple birth (%)	14 (16.9)	18 (22.8)
Outborn babies (%)	24 (28.9)	23 (29.1)
Low socioeconomic status (%)	30 (36.1)	40 (50.6)
Respiratory disorders (%)	68 (81.9)	76 (96.2)
ICH/IVH (%)	18 (21.7)	11 (13.9)
BPD (%)	14 (16.9)	10 (12.7)
NEC (%)	13 (15.7)	10 (12.7)

BPD, bronchopulmonary dysplasia; ICH, intracranial hemorrhage; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity.

**Table 3** Distribution of ROP severity based on birth weight and gestational age

Variable		Cases (n=83)		
		Type 1 ROP (n=26)	Type 2 ROP (57)	Control (n=79)
Birth weight (g)	<1000 (%)	13 (50)	11 (19)	11 (14)
	1000–1500 (%)	13 (50)	34 (59)	46 (58)
	>1500 (%)	0 (0)	12 (21)	22 (28)
Gestational age (weeks)	<28 (%)	12 (46)	3 (5)	8 (10)
	28–32 (%)	9 (35)	34 (60)	44 (56)
	>32 (%)	5 (19)	20 (35)	27 (34)

ROP, retinopathy of prematurity.



**Table 4** Results of the PCR-RFLP and PCR-SSP analyses

ND gene polymorphism/ mutation	Restriction enzymes	Size of PCR product/ND gene	Size after cutting	PCR-RFLP results (n=162)			
				Genotype	Type 1 ROP (n=26)	Type 2 ROP (n=57)	Control (n=79)
C597A	HaeIII	297 bp	CC: 12, 68, 104, 113 bp	CC	26	57	79
			CA: 12, 68, 104, 113, 172 bp	CA	0	0	0
			AA: 12, 113, 172 bp	AA	0	0	0
L108P	HaeIII		LL: 12, 68, 104, 113 bp	LL	26	57	79
			LP: 12, 30, 63, 83, 104, 113 bp	LP	0	0	0
			PP: 12, 30, 68, 83, 104 bp	PP	0	0	0
R121W	MspI		RR: 58, 72, 169 bp	RR	26	57	79
			RW: 58, 72, 130, 169 bp	RW	0	0	0
			WW: 130, 169 bp	WW	0	0	0
A105T	MbolI		AA: 297 bp	AA	26	57	79
			AT: 103, 194, 297 bp	AT	0	0	0
			TT: 103, 194 bp	LL	0	0	0
V60E	Not all optimisations for primers on PCR-SSP can be performed.						
C110G							

ND, Norrie disease; PCR-RFLP, PCR-restriction fragment length polymorphism; PCR-SSP, PCR-single specific primer; ROP, retinopathy of prematurity; bp, base pair.

RefSeqGene on chromosome X with an access code NG 009832.1. Furthermore, the sequences were aligned with GenBank's sequences using ClustalW multiple alignment program. We found no differences in the arrangement of bases compared with the BLAST of the ND gene in six mutation sites, that is, C597A, L108P, R121W, A105T, V60E and C110G, in either cases or controls, in exon 3 (table 5).

## DISCUSSION

We did not find any mutations in the ND gene in the 83 infants with ROP as well as in the 79 infants in the control group. This result does not support the hypothesis that this gene may be involved in the development or progression of ROP in infants from Indonesia. Our result cannot explain why severe ROP is more frequent in Indonesian infants than in infants from other racial backgrounds.

There are two explanations for our findings. First, there might be variations in the presence of mutations among different countries in Asia. Our results are in line with a study from Korea which also found no mutations in the

Norrie gene in infants with ROP.<sup>9</sup> A study from Hiraoka *et al*<sup>12</sup> found one infant with a heterozygous mutation in the 5' untranslated region (UTR) of exon 1 ND gene in 17 Japanese infants with advanced ROP and an insertion in the signal peptide of LRP5 in another infant.<sup>12</sup> In another study Hiraoka *et al*<sup>11</sup> failed to find mutations in exons 2 and 3 and the 3' UTR of the ND gene. They found in 100 patients with severe ROP two different mutations in exon 1.<sup>11</sup> We did not study these mutations because these mutations were reported only once. Further studies are needed to confirm this possibility.

Second, previous studies have shown inconsistent results regarding the mutations in the ND gene and the development or progression of ROP.<sup>6-8 10 11 19 27 28</sup> Shastri *et al*<sup>6</sup> described missense mutations (R121W and L108P) in 4 out of 16 infants with stage 4 or 5 ROP. In a follow-up study, they found one patient with an insertion in exon 1 and one with a deletion in the same exon in 100 infants with ROP.<sup>11</sup> Haider *et al*<sup>7</sup> initially failed to find a relation between mutations in R121W and L108P in infants from Kuwait. Later, he found a polymorphism in the ND

**Table 5** Multiple alignment results of ND gene sequences from samples and ND gene sequences from GenBank

ND gene polymorphism/mutation	DNA sequencing (n=36)			
	Genotype	Type 1 ROP (n=17)	Type 2 ROP (n=9)	Control (n=10)
C597A	CC	17	9	10
	CA	0	0	0
	AA	0	0	0
L108P	LL	17	9	10
	LP	0	0	0
	PP	0	0	0
R121W	RR	17	9	10
	RW	0	0	0
	WW	0	0	0
A105T	AA	17	9	10
	AT	0	0	0
	TT	0	0	0
V60E	VV	17	9	10
	VG	0	0	0
	GG	0	0	0
C110G	CC	17	9	10
	CG	0	0	0
	GG	0	0	0

ND, Norrie disease; ROP, retinopathy of prematurity.

gene C597A in 23 out of 24 infants with advanced stage ROP.<sup>19</sup> Talks *et al*<sup>27</sup> found two patients with a deletion in exon 1 of the ND gene in a group of 22 patients with stage 4 or 5 ROP. On the other hand, Dickinson *et al*<sup>10</sup> found no increased incidence of ND mutations in infants with ROP. Hutcheson *et al*<sup>28</sup> found five novel nucleotide changes in 143 infants with severe ROP, one in the 5' UTR region of exon 2 and four in the 3' UTR region of exon 3. They concluded that ND gene polymorphism might play a role in the pathogenesis of ROP, but does not appear to be a major causative factor. Recently, Dailey *et al*<sup>8</sup> found an FZD4 gene mutation, instead of mutations of the ND gene, to be associated with severe ROP. Hartnett<sup>29</sup> recently noted a lack of consensus in reports of genetic variants associated with ROP, including VEGF, EPAS1, SOD and the WNT signalling pathway as well as the ND gene. Hartnett *et al*<sup>30</sup> reported a large study of two cohorts (817 and 543 infants) of US preterm infants with birth weight <1000 g and found an single nucleotide polymorphism (SNP) in the BDNF gene that encodes for brain-derived neurotrophic factor to be significantly associated with severe (threshold) ROP. No mutations in the ND gene were found. We also did not find any mutations in the ND gene in the infants with ROP.

Finally, DNA sequencing was performed on 36 infants. The samples were selected based on a simple random sampling method. The purpose of the sequencing was to

detect polymorphisms in all mutations, including V60E and C110G. The  $\chi^2$  goodness of fit was used to identify the consistency between the characteristics of the 36 infants and those of the whole group. All variables showed a p value of >0.05, indicating that there were no significant differences between these 36 infants and the whole group. It can be concluded that the 36 samples are representative of the whole group.

A limitation of our study is that we limited the search for mutations in the ND gene to mutations that were described more than once in the literature. We realise that we may have missed mutations, mutations that might be found especially in infants in Asia. More studies will be needed to screen for more mutations than we did.

Based on our results and previously published studies, we conclude that it is very unlikely that the six polymorphisms in exon 3 of the ND gene studied in this paper are involved in the development or progression of ROP. Further studies must be conducted to find definitive explanations as to why ROP regresses in some patients, while it progresses in others.

**Acknowledgements** This work was supported by Harapan Kita Women and Children Hospital, Jakarta Eye Center, and Budi Kemuliaan Hospital. We would like to thank all doctors, especially Nani H Widodo MD and Florence Manurung MD, who conducted the ophthalmological examination of premature babies we studied in this research.

**Contributors** JES is the person who wrote the first draft of this manuscript and is involved directly in major aspects of this research/manuscript. SR developed and designed the study and helped in writing the report. RSS and IS assisted in data analysis and contributed to the interpretation and discussions in the ophthalmology and genetics sections. AS conducted the statistical analysis and worked on the report. PJS assisted in literature review and assisted in writing the paper.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

**Patient consent in publication** Not required.

**Ethics approval** The study was approved by the Institutional Review Board of the University of Indonesia School of Public Health. Informed consent was obtained from parents of all infants.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

## REFERENCES

- Gilbert C, Awan H. Blindness in children - half of it is avoidable, and suitable cost effective interventions are available. *Br J Ophthalmol* 2003;327:760-1.
- Larsson Eet *al*. Incidence of ROP in two consecutive Swedish population based studies. *Br J Ophthalmol* 2002;86:1122-6.
- Edy Siswanto J, Sauer PJ. Retinopathy of prematurity in Indonesia: incidence and risk factors. *J Neonatal Perinatal Med* 2017;10:85-90.
- Zin A, Gole GA. Retinopathy of prematurity-incidence today. *Clin Perinatal* 2013;40:185-200.
- Bizzarro MJ, Hussain N, Jonsson B, *et al*. Genetic susceptibility to retinopathy of prematurity. *Pediatrics* 2006;118:1858-63.
- Shastri BSet *al*. Identification of missense mutations in the Norrie disease gene associated with advanced retinopathy of prematurity. *Arch Ophthalmol* 1997;115:651-5.

7. Haider MZ, Devarajan LV, Al-Essa M, *et al.* Missense mutations in Norrie disease gene are not associated with advanced stages of retinopathy of prematurity in Kuwaiti Arabs. *Neonatology* 2000;77:88–91.
8. Dailey WA, Gryc W, Garg PG, *et al.* Frizzled-4 variations associated with retinopathy and intrauterine growth retardation: a potential marker for prematurity and retinopathy. *Ophthalmology* 2015;122:1917–23.
9. Kim JH, Yu YS, Kim J, *et al.* Mutations of the Norrie gene in Korean ROP infants. *Korean J Ophthalmol* 2002;16:93–6.
10. Dickinson JL, Sale MM, Passmore A, *et al.* Mutations in the NDP gene: contribution to Norrie disease, familial exudative vitreoretinopathy and retinopathy of prematurity. *Clin Exp Ophthalmol* 2006;34:682–8.
11. Hiraoka M, Berinstein DM, Trese MT, *et al.* Insertion and deletion mutations in the dinucleotide repeat region of the Norrie disease gene in patients with advanced retinopathy of prematurity. *J Hum Genet* 2001;46:178–81.
12. Hiraoka M, Takahashi H, Orimo H, *et al.* Genetic screening of Wnt signaling factors in advanced retinopathy of prematurity. *Molecular Vision* 2010;16:2572–7.
13. Cooke RW, Drury JA, Mountford R, *et al.* Genetic polymorphisms and retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 2004;45:1712–5.
14. Mohamed S, Schaa K, Cooper ME, *et al.* Genetic contributions to the development of retinopathy of prematurity. *Pediatr Res* 2009;65:193–7.
15. Xu Q, Wang Y, Dabdoub A, *et al.* Vascular development in the retina and inner ear: control by norrin and frizzled-4, a high-affinity ligand-receptor pair. *Cell* 2004;116:883–95.
16. Cryotherapy for Retinopathy of Prematurity Cooperative Group. Multicenter trial of cryotherapy for retinopathy of prematurity. One-year outcome - structure and function. *Arch Ophthalmol* 1990;108:1408–16.
17. Saunders RA, Donahue ML, Christmann LM, *et al.* Racial variation in retinopathy of prematurity. The cryotherapy for retinopathy of prematurity Cooperative Group. *Arch Ophthalmol* 1997;115:604–8.
18. William V. On behalf of the early treatment for retinopathy of prematurity Cooperative Group. final results of the early treatment for retinopathy of Prematurity (ETROP) randomized trial. *Trans Am Ophthalmol Soc* 2004;102:233–50.
19. Haider MZ, Devarajan LV, Al-Essa M, *et al.* A C597A polymorphism in the Norrie disease gene is associated with advanced retinopathy of prematurity in premature Kuwaiti infants. *J Biomed Sci* 2002;9:365–70.
20. Meindl A, Berger W, Meitinger T, *et al.* Norrie disease is caused by mutations in an extracellular protein resembling C-terminal globular domain of mucins. *Nat Genet* 1992;2:139–43.
21. Torrente I, Mangino M, Gennarelli M, *et al.* Two new missense mutations (A105T and C110G) in the norrin gene in two Italian families with Norrie disease and familial exudative vitreoretinopathy. *Am J Med Genet* 1997;72:242–4.
22. Haider MZ, Devarajan LV, Al-Essa M, *et al.* Retinopathy of prematurity: mutations in the Norrie disease gene and the risk of progression to advanced stages. *Pediatr Int* 2001;43:120–3.
23. Wheatley CM *et al.* Retinopathy of prematurity: recent advances in our understanding. *Br J Ophthalmol* 2002;86:696–700.
24. Schuback DE, Chen ZY, Craig IW, *et al.* Mutations in the Norrie disease gene. *Hum Mutat* 1995;5:285–92.
25. Meindl A, Lorenz B, Achatz H, *et al.* Missense mutations in the NDP gene in patients with a less severe course of Norrie disease. *Hum Mol Genet* 1995;4:489–90.
26. Ott S, Patel RJ, Appukuttan B, *et al.* A novel mutation in the Norrie disease gene. *J Aapos* 2000;4:125–6.
27. Talks SJ *et al.* De novo mutations in the 5' regulatory region of the Norrie disease gene in retinopathy of prematurity. *J Med Gen* 2001;38:e46:46e–46.
28. Hutcheson KA, Paluru PC, Bernstein SL, *et al.* Norrie disease gene sequence variants in an ethnically diverse population with retinopathy of prematurity. *Mol Vis* 2005;11:501–8.
29. Hartnett ME. Advances in understanding and management of retinopathy of prematurity. *Surv Ophthalmol* 2017;62:257–76.
30. Hartnett ME, Morrison MA, Smith S, *et al.* Genetic variants associated with severe retinopathy of prematurity in extremely low birth weight infants. *Invest Ophthalmol Vis Sci* 2014;55:6194–203.
31. Buffenn AN, Bernstein SL, Hutcheson KA. Identification of a new Norrie disease gene mutation in an African-American infant with advanced retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 1999;40:S565.